Kim, J. H. & Miller, L. L. (1969) *J. Biol. Chem.* **244**, 1410

Moir, A. T. B. (1971) *Brit. J. Pharmacol.* **43**, 724

Powanda, M. C. & Wannemacher, R. W. (1971) *Biochim. Biophys. Acta* **252**, 239

Rapoport, M. I., Feigin, R. D., Bruton, J. & Beisel, W. R. (1966) *Science* **153**, 1642

Ross, D. S., Fernstrom, J. D. & Wurtman, R. J. (1972) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **31**, 900

Scapagnini, U., Preziosi, P. & De Schaepdryver, A. (1969) *Pharmacol. Res. Commun.* **1**, 63

Sourkes, T. L., Missala, K. & Oravec, M. (1970) *J. Neurochem.* **17**, 111

The Effects of Drugs on the Activities of 5-Aminolaevulinate Synthetase and other Enzymes in the Pathway of Haem Biosynthesis

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It has been known for some time that certain drugs can alter the regulation of haem biosynthesis in the liver of the experimental animal and cause a condition of hepatic porphyria, characterized by the accumulation of the intermediates of the pathway that are the porphyrins and their precursors, 5-aminolaevulinate and porphobilinogen (reviewed by Tschudy & Bonkowsky, 1972). A very important achievement in the elucidation of the mechanism of induction of porphyria by drugs was the finding by Granick & Urata (1963) that the activity of liver 5-aminolaevulinate synthetase was markedly increased in experimental porphyria caused by 3,5-diethoxycarbonyl-1,4-dihydrocollidine, a finding later extended to hepatic porphyrias caused by 2-allyl-2-isopropylacetamide (Marver *et al.*, 1966) and griseofulvin (Nakao *et al.*, 1967). This enzyme appears to be the rate-limiting step in the overall pathway and also the site where haem, the end product, exercises a feedback control (reviewed by Granick & Sassa, 1971). The activities of the two subsequent enzymes in the pathway, 5-aminolaevulinate dehydratase and porphobilinogen deaminase, have also been reported to be increased in the liver of animals rendered porphyric with drugs (Gibson *et al.*, 1955; De Matteis & Gibbs, 1972; Miyagi *et al.*, 1971; Strand *et al.*, 1970); these increases are, however, relatively small and may be secondary to the stimulation of the synthetase and to the consequent accumulation of 5-aminolaevulinate and porphobilinogen in the liver (cf. Onisawa & Labbe, 1962).

The mechanism by which drugs stimulate the activity of liver 5-aminolaevulinate synthetase is not yet known. Since the enzyme is subject to feedback control by haem, a likely possibility is that the drugs increase the activity of the enzyme by interfering in some way with this feedback control. A clear distinction must be made between drugs that increase markedly the activity of the enzyme and induce porphyria and drugs that cause only a small stimulation of the enzyme without a significant accumulation of porphyrins in the liver. Examples of drugs of the first group are the porphyrogenic compounds 2-allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine and griseofulvin. A transient decline in the concentration of cytochrome *P*-450 and haem is observed in the liver microsomal fraction after administration of any of these drugs to the experimental animal, coincidental with the rise in activity of 5-aminolaevulinate synthetase. This suggests that the porphyrogenic drugs may stimulate markedly the activity of the enzyme by lowering the concentration of haem in the liver, thereby diminishing the normal feedback control.

With 2-allyl-2-isopropylacetamide the loss of liver haem is due to increased destruction and conversion into certain unidentified green pigments (De Matteis, 1971; Meyer & Marver, 1971). The allyl group in the molecule of the drug appears to be important for both destruction of liver haem and stimulation of 5-aminolaevulinate synthetase activity, possibly after conversion, by way of drug metabolism, into a reactive derivative (Abbritti & De Matteis, 1971–72). Although the microsomal fraction is the main site of haem destruction, a loss of radioactivity is also observed from rapidly labelled haem in the cytosol and in the crude mitochondrial fraction of the liver homogenate of treated animals: this may be relevant to the finding (Hayashi *et al.*, 1969) that a stimulation of the synthetase is observed after the administration of 2-allyl-2-isopropylacetamide in the cytosol as well as in the mitochondria.

With 3,5-diethoxycarbonyl-1,4-dihydrocollidine there is probably some degree of liver haem destruction, but inhibition of liver haem synthesis may also be important in causing the loss of liver haem and the stimulation of 5-aminolaevulinate synthetase activity. A rapid inhibition of the chelatase activity is caused by the drug in liver mitochondria, before any increase in the activity of the synthetase becomes apparent (De Matteis & Gibbs, 1972); mice, which are more sensitive than rats to the stimulation of the synthetase by this drug, are also more sensitive to the inhibition of the chelatase (F. De Matteis, G. Abbritti & A. Gibbs, unpublished work). Inhibition of haem synthesis may also be important in the stimulation of 5-aminolaevulinate synthetase by griseofulvin. Lockhead *et al.* (1967) reported an inhibition of the incorporation *in vivo* of ^{59}Fe into liver haem in mice made porphyric by feeding with griseofulvin for 2 weeks. I have now found that griseofulvin causes an early inhibition of the mitochondrial chelatase, before a marked rise in the activity of the synthetase becomes apparent; also, the 2'-β-hydroxyethyl thio-

ether analogue of griseofulvin, which is not porphyrogenic, does not inhibit the activity of the chelatase.

The stimulation of 5-aminolaevulinate synthetase activity by both 2-allyl-2-isopropylacetamide and 3,5-diethoxycarbonyl-1,4-dihydrocollidine can be prevented by prior administration to the rat of either compound SKF 525-A (2-diethylaminoethyl 3,3-diphenylpropylacetate) or cycloheximide. An important difference is that compound SKF 525-A also prevents the destruction of liver haem by 2-allyl-2-isopropylacetamide and the inhibition of the chelatase by 3,5-diethoxycarbonyl-1,4-dihydrocollidine, whereas cycloheximide does not (De Matteis, 1971; F. De Matteis, G. Abbritti & A. Gibbs, unpublished work). The significance of these findings and the possible mechanism of action of the two inhibitors will be discussed in the light of the hypothesis (Granick, 1966) that the stimulation of the synthetase caused by drugs involves synthesis *de novo* of the enzyme protein.

In clear contrast with the porphyrogenic compounds considered so far, a second group of drugs, including phenobarbitone and phenylbutazone, increases rather than decreases the concentration of cytochrome P-450 and haem in the microsomal fraction without causing accumulation of porphyrins in the liver. The small stimulation of 5-aminolaevulinate synthetase activity caused by these drugs possibly reflects an increased haem utilization rather than an interference with the regulation of the pathway. However, when either phenobarbitone or phenylbutazone is given together with a small dose of 3,5-diethoxycarbonyl-1,4-dihydrocollidine, the stimulation of 5-aminolaevulinate synthetase activity caused by this latter drug is greatly enhanced (De Matteis, 1972). The following interpretation is possible for this potentiation effect. 3,5-Diethoxycarbonyl-1,4-dihydrocollidine may, by inhibiting the synthesis of liver haem, decrease the concentration of haem available for control of the synthetase. Phenobarbitone and phenylbutazone, by promoting an accumulation of cytochrome P-450 apoprotein, may stimulate haem utilization and thereby decrease the concentration of this 'regulatory' haem still further.

Abbritti, G. & De Matteis, F. (1971–72) *Chem.-Biol. Interact.* 4, 281–286

De Matteis, F. (1971) *Biochem. J.* 124, 767–777

De Matteis, F. (1972) *Biochem. J.* 127, 21 P–22 P

De Matteis, F. & Gibbs, A. (1972) *Biochem. J.* 126, 1149–1160

Gibson, K. D., Neuberger, A. & Scott, J. J. (1955) *Biochem. J.* 61, 618–629

Granick, S. (1966) *J. Biol. Chem.* 241, 1359–1375

Granick, S. & Sassa, S. (1971) *Metab. Pathways* 5, 77

Granick, S. & Urata, G. (1963) *J. Biol. Chem.* 238, 821–827

Hayashi, N., Yoda, B. & Kikuchi, G. (1969) *Arch. Biochem. Biophys.* 131, 83–91

Lockhead, A. C., Dagg, J. H. & Goldberg, A. (1967) *Brit. J. Dermatol.* 79, 96–102

Marver, H. S., Collins, A., Tschudy, D. P. & Rechcigl, M. (1966) *J. Biol. Chem.* 241, 4323–4329

Meyer, U. A. & Marver, H. S. (1971) *Science* 171, 64–66

Miyagi, K., Cardinal, R., Bossenmaier, I. & Watson, C. J. (1971) *J. Lab. Clin. Med.* 78, 683–695

Nakao, K., Wada, O., Takaku, F., Sassa, S., Yano, Y. & Urata, G. (1967) *J. Lab. Clin. Med.* 70, 923–932

Onisawa, J. & Labbe, R. F. (1962) *Biochim. Biophys. Acta* 56, 618–620

Strand, L. J., Felsher, B. F., Redeker, A. G. & Marver, H. S. (1970) *Proc. Nat. Acad. Sci. U.S.* 67, 1315–1320

Tschudy, D. P. & Bonkowski, H. L. (1972) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* 31, 147–159

Mechanisms and Consequences of the Induction of Microsomal Enzymes of Mammalian Liver

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The mixed-function oxidase system present in the endoplasmic reticulum of mammalian liver is responsible, through its terminal oxygenase cytochrome P-450, for the oxidative metabolism of cholesterol, steroids, fatty acids, drugs and other xenobiotics (Parke, 1968). The induction of this enzyme system has been widely studied and may be effected by a great diversity of drugs, steroids, carcinogens and other foreign compounds (Conney, 1967; Parke, 1971a). Several theoretical models for translational and transcriptional control of the relevant gene expression have been examined (Venkatesan *et al.*, 1971). An important criterion for induction, at least at the translational level, would appear to be that the inducing agent is a substrate of this microsomal enzyme system. One of the major physiological roles of this enzyme system is undoubtedly the oxidative metabolism of anutrient xenobiotics of the diet, thus facilitating their elimination from the body. It might therefore be expected that natural dietary anutrients would also be effective inducing agents of the microsomal mixed-function oxidase system, and a number of such compounds, such as β-ionone and safrole, have been shown to induce these enzymes (Parke & Rahman, 1969, 1970). If enzyme induction is primarily an adaptive process, it might be expected that this most versatile enzyme system with its wide diversity of substrates would be among the foremost of mammalian enzymes to manifest induction, and, with the present widespread use of synthetic chemicals such as drugs, food additives, pesticides etc., this induction would have high potential and might exhibit certain abnormalities.

One of the most widely studied inducing agents of this enzyme system is the barbiturate phenobarbital. Studies of the inducing potential of a series of barbiturates, including pentobarbital, secobarbital, allobarbital, thiopental and phenobarbital, have shown